

wish to thank the members of the St. Paul's Hospital Biochemistry Laboratory who performed all the acid phosphatase determinations, and the departments of medical illustration and photography for the preparation of the Chart.

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Preliminary Communications

Endotoxin and Acute Renal Failure Associated with Obstructive Jaundice

British Medical Journal, 1970, **4**, 472-474

Summary: A single dose of endotoxin given to rats with obstructive jaundice produced death with intravascular coagulation. This action was apparently due to delayed clearance of endotoxin from the circulation. The finding is relevant to "hepatorenal failure," which can be caused by bacteraemia after biliary tract operations.

INTRODUCTION

About 12% of patients who undergo operations for biliary tract disease develop acute renal failure (Funck-Brentano, Méry, Vantelon, and Watchi, 1963). Morphological changes range from mild to severe tubular necrosis (Bloodworth and Sommers, 1959) but occasional patients have renal cortical necrosis. Most clinicians agree that a Gram-negative septicæmia often follows such surgery (Caroli, 1958; Crosnier, 1958; Hamburger, 1958; Hepp, 1958), so that some cases of acute renal failure with obstructive jaundice could be the result of endotoxic shock. Such cases would be the human equivalent of a Shwartzman reaction (Hjort and Rapaport, 1965), and this concept is particularly relevant to the development of cortical necrosis (Vassalli and Richet, 1961).

We noticed that a single dose of endotoxin given to the rat with obstructive jaundice usually caused death with much fibrin deposition in the renal vasculature. Investigation shows that the clearance of endotoxin from the blood stream is delayed in the presence of obstructive jaundice. Endotoxin is normally removed from the blood by the phagocytic cells of the liver and spleen (Braude, Carey, and Zalesky, 1955).

METHODS

Obstructive jaundice was produced in the rat by tying the common bile duct just before entry into the duodenum. Three days later these rats and appropriate controls were given *Escherichia coli* endotoxin 0127:B8 (Difco) by intravenous injection in a dose of 100 µg./100 g. body weight. This is a large dose of endotoxin. Coagulation studies included the measurement of prothrombin time and the thrombin-clotting time, the platelet count, and the plasma fibrinogen, together with fibrin monomer. Fibrin monomer is the initial hydrolytic product of the action of thrombin on fibrinogen—that is, fibrinogen with peptides A and B removed—and is normally polymerized by the fibrin-stabilizing factor. Fibrin monomer can be estimated by aggregation with protamine sulphate (Lipiński and Warowski, 1968).

Rats given rat fibrinogen prepared and labelled with 131-iodine by the method of McFarlane (Campbell *et al.*, 1956) were studied. The half-life of the labelled fibrinogen and the shape of the disappearance curve were determined from serial plasma fibrinogen counts. In interpreting the curves of plasma fibrinogen survival it should be noted that a large dose of thromboplastin causes "absolute defibrination" with a

profound permanent loss of labelled fibrinogen, but a small dose can cause "rebound defibrination," in which, after an immediate depletion of labelled fibrinogen, from the intravascular pool, there is a later reappearance of labelled material (Adelson, 1968). This further fibrinogen probably returns from the extravascular space by way of the lymphatics (E. N. Wardle, unpublished observations).

Studies were performed on groups of four rats, two normal and two with jaundice. On the third day of the study they were given the intravenous endotoxin. Some also received intravenous Isothrodym (neodym-3-sulpho-isonicotinate) at a dose of 50 mg./kg. weight. This rare earth metal derivative was originally found to have an anticoagulant action, but is now known to produce reticuloendothelial blockade (Lázár, Karády, and Husztik, 1969) and to enhance intravascular coagulation (Lázár and Karády, 1965). In further studies the clearance of chromium-labelled endotoxin (Herring, Herion, Walker, and Palmer, 1963) or of microaggregated 131-iodine-labelled human albumin (Shaldon, Chiandussi, Guevara, Caesar, and Sherlock, 1961) was determined by collecting timed samples from the rat tail vein at intervals after intravenous injection. Microaggregated albumin was used in a dose of 0.5 mg./100 g. body weight (Benacerraf, Biozzi, Halpern, Stiffel, and Mouton, 1957), and for each batch normal clearance times were determined. Histological sections were stained with haematoxylin and eosin and Martius scarlet-blue for fibrin (Lendrum, Fraser, Slidders, and Henderson, 1962).

RESULTS

Of animals with obstructive jaundice given a single dose of endotoxin 18 out of 20 animals died, whereas only 2 out of 10 normal animals died. Those animals which died did so within a period of four to six hours.

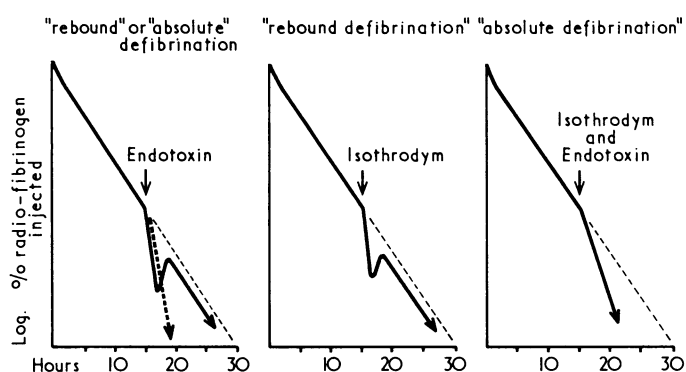
A synopsis of the coagulation studies is given in Table I, where mean values for six pairs of animals before and after the injection of a single dose of endotoxin are given. Blood samples were collected 10 minutes after the dose, either from the tail vein or by cardiac puncture.

The results of the radioactive fibrinogen catabolism studies are shown in the Chart. One-third of the animals with jaundice given a single dose of endotoxin showed "rebound defibrination." In about another third there appeared to be "absolute defibrination," but death was sometimes too early to allow for serial specimens. Animals with obstructive jaun-

TABLE I.—Coagulation Studies before and after a Single Dose of Endotoxin

	Normal Animals		Obstructive Jaundice	
	Before	After	Before	After
Prothrombin (seconds) ..	10.0	90.0	120	300
Thrombin clotting-time (seconds)	5.0	7.0	3.0	30.0
Platelets (per mm. ³) ..	390,000	350,000	380,000	160,000
Fibrinogen (mg./100 ml.) ..	290	260	400	300
Fibrin monomer (optical density units) ..	0.14	0.12	0.105	0.105
Fibrinogen half-life (hours) ..	20—40		18.0—30.0	

Mean values for six pairs of animals.



Radio-fibrinogen catabolism with endotoxin in obstructive jaundice.

dice had a somewhat higher plasma fibrinogen than normal controls and also sometimes a shorter half-life even without administration of endotoxin. The injection of Isothrodym also caused "rebound defibrination," and Isothrodym combined with endotoxin produced "absolute defibrination" with death.

Since these results show sensitivity of the animal with obstructive jaundice to endotoxin, clearances of chromium-labelled endotoxin were performed and compared with clearances of microaggregated ¹³¹-iodine-labelled human albumin. These results are summarized in Table II. The effect of a single dose of endotoxin on the clearance rate of microaggregated albumin is shown in Table III.

TABLE II.—Clearance Studies

	Six Normal Animals	Six Obstructive Jaundice	
Cr-endotoxin . .	6.5—8.5 min.	11.0—30.0 min.	$0.001 < P < 0.01$ $t = 3.8$ (10 D.F.) $P < 0.001$ $t = 4.6$ (10 D.F.)
Microaggregated albumin . .	10.0—12.0 min.	12.0—16.0 min.	

The half-time of the plasma clearance is given in each case. Statistical analysis using Student's *t* test and Bessel's correction.

TABLE III.—Clearance Studies of Microaggregated Albumin in Four Animals with Obstructive Jaundice

Before Endotoxin		After Endotoxin
9.0 minutes	10.0 minutes
8.0 "	11.0 "
9.0 "	11.0 "
9.0 "	12.5 "

The same effect is also found in normal animals.

The presence of biliary obstruction was later confirmed histologically. The animals with obstructive jaundice only showed early "toxic" renal tubular degenerative changes. These animals also showed more stasis fibrin in the renal vasculature as compared with control animals. The rats with obstructive jaundice who were given endotoxin showed massive fibrin deposition in the renal vasculature. The glomerular capillaries were largely spared, but the interlobular arteries, the intertubular capillaries, and vasa rectae were filled with Martius scarlet-blue positive material. These changes are usually seen as a prelude to the development of renal cortical necrosis, but the animals died before recognizable nuclear degenerative changes became apparent. These endotoxin-treated animals also showed deposition of fibrin in the hepatic sinusoids. Death was thought to be due to a cardiotoxic action of the endotoxin.

Animals with jaundice given Isothrodym alone also showed fibrin in the renal vasculature and hepatic sinusoids, while endotoxin and Isothrodym combined produced most pronounced fibrin deposition.

DISCUSSION

The fact that a single dose of endotoxin will cause a Shwartzman reaction in the animal with obstructive jaundice is clearly important to the understanding of the "hepatorenal syndrome." Normally two spaced doses of endotoxin are required to elicit the reaction in the rabbit and to produce the same functional phenomenon in the rat (unpublished

observations). The rat does tend to develop liver cell necrosis (Gronvall and Brunson, 1956). Known ways of "preparing" for the Shwartzman reaction are induction of pregnancy (Kliman and McKay, 1958), administration of cortisone (Thomas and Good, 1951), injection of fat (McKay, Margaretten, and Rothenberg, 1964; Huth, Schoenborn, and Knorpp, 1967), reticuloendothelial blockade (Good and Thomas, 1952), or inhibition of fibrinolysis (Lee, 1962). Reticuloendothelial blockade with Thorotrast is an experimental technique used to demonstrate intravascular coagulation (Lerner, Rapaport, and Spitzer, 1968), but we have used Isothrodym. The final result of intravascular coagulation in the rabbit is cortical necrosis; in the rat some renal deposition of fibrin and tubular damage can be found by sacrifice of the animals, but death is unusual.

Our further studies show that the animal with obstructive jaundice develops intravascular coagulation because the clearance of endotoxin is delayed. This could be because there is already some liver cell necrosis in the animal with obstructive jaundice (Farrar, Eidson, and Kent, 1968; Nolan and Ali, 1968), but probably liver perfusion is also slightly reduced after endotoxin as shown by the reduced clearance of microaggregated albumin (Tables II and III). Of greater magnitude is the slowing of the reticuloendothelial clearance of endotoxin (Table II). Eventually, however, the uptake of endotoxin by the liver and spleen is quantitatively the same as in normal animals. It is therefore unlikely to be due to a deficiency of opsonins (Chedid, Parant, and Parant, 1970), but the refractoriness of the reticuloendothelial system may be an effect of metabolites normally excreted in the bile. In addition, endotoxin itself is known to paralyse phagocytic cells (Benacerraf and Sebestyen, 1957), an effect which is dose-dependent, and the liver of obstructive jaundice might be more susceptible.

The other major effect of endotoxin is the vasoconstrictive action, and in the dog it has been shown (Weil, MacLean, Visscher, and Spink, 1956) that constriction of the hepatic veins produces pooling of blood in the portovenous system. As shown in Table III a vasoconstrictor effect could explain a moderate delay in the clearance of aggregated albumin, which would account for the values given in Table II for albumin, but the delay of clearance of labelled endotoxin is greater. Clearly there is scope here for further study, particularly of the relevance to man.

Dawson (1968) has shown, firstly, that in obstructive jaundice there is an association between the depth of jaundice and the onset of anuria, and, secondly, that the kidney of a rat with obstructive jaundice will not survive a clamp on the renal artery for a limited period of only 60 minutes. It has also been shown that toxicity is due to the effect of bilirubin glucuronide (Baum, Stirling, and Dawson, 1969). On the results of our findings we think that bacteraemia may have particular significance in the genesis of some cases of acute renal failure in jaundice.

We thank Professor D. N. S. Kerr and Professor A. G. Heppleston for support. E. N. W. gratefully acknowledges a grant from the Medical Research Council.

E. N. WARDLE, M.B., M.R.C.P.,
M.R.C. Clinical Research Fellow.

N. A. WRIGHT, M.B.,
University Demonstrator in Pathology.

Royal Victoria Infirmary,
Newcastle upon Tyne NE1 4LP.

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Medical Memoranda

Neuropathy, IgM Paraproteinaemia, and Autoantibodies in Hypernephroma

British Medical Journal, 1970, 4, 474

In a recent review of 72 patients (Croft and Wilkinson, 1969) neurological symptoms preceded the finding of malignancy in 49%, the mean interval before discovery of the neoplasm being two years. In the case reported here neurological symptoms preceded diagnosis of hypernephroma by four and a half years.

CASE REPORT

A 58-year-old timber salesman had been in good health until 1964, when he developed weakness of his left hand associated with numbness of the ring and little fingers. A few weeks later he noticed weakness of his right foot and intermittent paraesthesia over the dorsum of his left foot.

On examination he was normotensive and had a left ulnar nerve palsy, a right lateral popliteal palsy, absent reflexes in the legs, and flexor plantar responses; vibration sense was diminished at both ankles. The clinical signs were those of mononeuritis multiplex in the context of a more generalized peripheral neuropathy. All investigations performed at that time were normal.

In July 1967 he was admitted to hospital for further assessment. Apart from the development of wasting, his physical signs remained unchanged, but investigations showed several new features. His E.S.R. had risen to between 42 and 60 mm. in the first hour and the C.S.F. protein to 80 mg./100 ml. All other investigations, including biopsy of the right sural nerve and underlying muscle, were normal.

During the next 18 months there was progressive deterioration with increasing weakness and general malaise. Examination in October 1968 disclosed bilateral wasting of the interossei muscles and hypothenar eminences associated with generalized weakness of the arms, most pronounced distally. There was global wasting of the right leg with gross distal weakness and associated foot drop. Power in the left leg was normal. All the reflexes were absent except the left triceps reflex, which was brisk; the left plantar response was extensor and the right plantar response produced fanning of the toes. A sensory loss in the territory of the ulnar nerve was present in both hands, and there was impairment of vibration and joint position sense at both ankles. Romberg's sign was positive and his gait ataxic.

Investigations.—Haemoglobin 112%; E.S.R. 68–71 mm. in the first hour; C.S.F. protein 70 mg./100 ml.—immunoelectrophoresis normal. Serum electrophoresis showed a non-specific increase in gammaglobulins—total globulin 4.1 g./100 ml.—and immunoelectrophoresis an IgM paraproteinaemia. IgG 1,280 mg./100 ml., IgM 74% of standard, IgA 136% of standard. Sternal marrow aspirate showed a hypercellular picture with evidence of iron deficiency; staining with fluorescent anti-human IgM showed no significant uptake of the antibody. The titre of organ-specific anti-brain

antibodies was 1:1,024. A barium-meal examination was performed. The films showed an enlarged left kidney, and a large vascular tumour in the lower pole was demonstrated by aortography.

Exploration was performed by Mr. Dawson Edwards and a large hypernephroma removed. At operation blood samples were taken from both renal artery and renal vein for immunoglobulin estimations; no significant difference was found. The tumour was also stained with fluorescent anti-human IgM, but no convincing uptake of the fluorescent stain occurred.

COMMENT

Though neurological syndromes associated with renal tumours have been previously described in the literature (Swan and Wharton, 1963; Madanagopalan and Saratchandra, 1966) this association is uncommon.

IgM paraproteinaemia has been reported in association with malignancy, and in this patient it was initially considered whether the paraprotein represented an antibody response to antigens in the hypernephroma. Immunofluorescent studies with the use of labelled anti-human IgM, however, failed to show any uptake of the patient's IgM by the tumour, and the IgM paraproteinaemia was thought to be of the essential monoclonal benign type (Waldenström, 1952).

Complement-fixing organ-specific anti-brain antibodies have previously been reported in four cases of sensory neuropathy and one with a neuromyopathic syndrome, all associated with carcinoma of the bronchus (Wilkinson, 1964). It is of interest that in this patient the autoantibodies were in the IgM fraction of the immunoglobulins and that they occurred in a high titre of 1:1,024. The IgM anti-brain antibodies could also be detected by immunofluorescence to a titre of 1:160. These titres, though significant, are not high enough to enable one to conclude that the IgM paraprotein was specifically directed against brain tissue. The relationship between the patient's neurological symptoms, autoantibodies, and hypernephroma remains obscure.

I should like to thank Dr. Michael Small for his encouragement in the preparation of this paper, and Dr. R. W. Thompson and Dr. N. Williamson for their interest and helpful advice in the immunological and immunofluorescent studies.

D. C. THRUSH, M.B., M.R.C.P.
Neurological Registrar, Queen Elizabeth Hospital
Birmingham 15.

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